

### **REMARKS**

Claim 40 is amended.

Claims 22-50 remain active and under consideration in this application.

### **REQUEST FOR RECONSIDERATION**

Claims 22-50 stand rejected under 35 USC 112, second paragraph, as ostensibly being indefinite.

In particular, the examiner considers that the terms 'may contain repair enzymes' and 'biological medium' are indefinite.

However, the suitable and definite nature of these terms is explained below.

- Claim 22, Step (d) // Repair Medium, i.e., "optionally containing enzyme activities for repair."

The examiner has at the first paragraph of page 3 of the current Official Action (OA) asked how the lesions in the target DNA molecule are repaired if the repair solution does not contain repair enzymes?

The purpose of the method according to the claimed invention is to ascertain the presence and effectiveness of excision and resynthesis DNA repair processes of an individual. To do this, as has been explained previously, a biological sample from an individual is combined with a repair

solution and allowed to act upon a DNA target which contains a number of lesions. The repair of these lesions is then ascertained and the DNA repair capacity of the individual is determined.

To specifically answer the examiner's question, therefore, if an individual does not possess functional DNA repair enzymes and the repair solution with which it is combined also does not contain DNA repair enzymes, then the DNA target will not be repaired. This lack of repair will provide information concerning the individual.

The reduced DNA repair capacity of an individual can result from a number of reasons, such as defective DNA repair enzymes, but also as a result of low levels of available ATP or other factors associated with the repair process. By providing a set of repair solutions which always contain an essential component of the claimed method, namely labelled nucleotide triphosphates, but which may also contain other components such as DNA repair enzymes, ATP, ATP regenerating systems; and by assaying each of these different combinations of the biological medium with a repair solution simultaneously the exact defect or defects leading to the individuals reduced DNA repair capacity can be determined in a single set of experiments.

- Biological Medium, i.e., claim 22, line 2 for example.

In the second paragraph on page 3 of the current Office Action, the examiner has questioned the meaning of the term "biological medium". In particular, the examiner notes that in other contexts a medium is a material such as agar or LB broth in which an organism can be inoculated and subsequently grow.

It is noted, however, that the definition of a "biological medium" according to the claimed invention is provided in the present specification at paragraph 24, as acknowledged by the examiner. It is well-established that Applicant may act as his/her own lexicographer and an explicit definition of this term has, accordingly, been provided. Alternatively, see page 4, lines 16-19 of the present specification as filed.

This biological medium maybe prepared in accordance with paragraph 100 of the present specification, that is according to the method set out in Manley et al., 1983, Methods Enzymol. 101, 568-582 or according to Biade et al., 1998, J. Biol. Chem., 273,898-942 or according to any other method which from a sample from an individual results in a biological medium which contains DNA repair proteins (if these are present in a functional form in the individual). Thus, this term is well-defined in the present specification. Further, page 15, lines 26-28 of the present specification as filed provides:

[The claimed method] is particularly suitable for studying various biological media and is a good reflection of the situation *in vivo*.

- Diagnosis of diseases associated with reduced DNA repair capacity

A number of important human diseases are associated with an individual having faulty DNA repair systems. These diseases include xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy.

Using the method according to claim 22, it is therefore possible to determine whether a pathology exhibited by the individual is due to (or not) a fault in the individual's DNA repair systems.

Applicant wishes to express her sincere gratitude to the examiner for withdrawing the previous ground of also rejection under 35 USC 102(b).

However, claims 22-25, 29, 31, 33, 38, 41-44, 47 and 50 stand rejected under 35 USC 103(a) as being unpatentable over US 2002/0022228 in view of Calsou et al.

Neither of these references, either alone or in combination with each other, would have disclosed or suggested the claimed invention to one skilled in the art at the time it was made.

In the current Office Action, the examiner has clearly accepted Applicant's previous arguments that '228 neither anticipates nor renders obvious the claimed invention even in combination with any of the previously cited prior art documents, because neither '228 nor any other previously cited document discloses or suggests the use of a mutated DNA target molecule which is supercoiled.

The examiner has alleged that this feature of a supercoiled DNA target is known from Calsou et al. and that the artisan would have arrived at the claimed method according to claim 22 by combining '228 and Calsou et al.

However, '228 and Calsou et al. are directly contrary to each regarding the nature of the DNA target.

Notably, '228 describes the use of short bound single or double stranded oligonucleotides (oligos) of known sequence which contain one or more defects such as a base mismatch or apurinic site, mimicking a mutation which requires excision and resynthesis repair. These oligos as well as the defects, also contain at their 5' or 3' end or in the 2' position of one of the deoxyribosyl residues a primary or secondary amino group, by which the oligo is covalently coupled via a squaric acid ester to a solid-phase matrix which also contains primary or secondary amino groups. The effects of excision and resynthesis repair mechanisms on these mutations are assayed by monitoring decreases in the level of detectable signal from the bound oligos, this decrease being associated with the repair of the defects as the oligos are acted upon they are cleaved from the matrix and hence their signal is lost. '228 describes that this signal can be assayed by the immunoblotting with labelled antibiotics directed against the DNA defects or by incorporating a detectable label into the oligos.

Calsou et al. describe the use of supercoiled and linear plasmid DNA, which have been treated so as to induce a number of random mutations. The effects of excision and resynthesis repair

mechanisms on these mutations are assayed by incubating in solution damaged and untreated supercoiled and linear plasmid DNA with radioactively labelled CTP and a cell extract. The DNA is then purified and linearized and run overnight on an agarose gel, following which the DNA products are visualized using ethidium bromide and UV. The quantification of the repair reaction is made by autoradiography, scintillation counting of excised bands and densitometry of the photographic negative of the gel so as to normalize the results based upon the efficiency of DNA recovery for each reaction sample.

Clearly, '228 and Calson et al. are, therefore, directly contradictory concerning the type of DNA target to be used in the quantification of the excision and resynthesis repair capacity of a biological sample. Neither reference discloses or suggests that using supercoiled plasmid DNA (in the case of '228) or ss or ds short oligonucleotides (in the case of Calson et al.) is desirable or would improve the existing methods described therein.

Thus, no reason could have existed for the artisan to combine the disclosures of these documents, hence, one skilled in the art would have no motivation to combine these references at all.

In the current Office Action, the examiner has phrased the question of how the skilled artisan would have arrived at a method according to claim 22, having reference to '228 and Calson et al., as "[t]he ordinary skilled artisan, desiring to perform a method for analyzing DNA repair by excision and resynthesis using supercoiled plasmids would have been motivated to combine the teachings of US 2002/0022228 {...} and Calson et al. {...}."

However, this phrasing of the how the artisan would have arrived at the claimed invention is based on an impermissible *ex post facto analysis* and application of cited prior art thereby. In particular, an important feature missing from '228 is that the DNA target contains supercoiled DNA. Clearly, *ex post facto analysis* and application of the cited prior art thereby remains impermissible even under KSR v. Teleflex, 550 U.S. 398 (2007). That is, the prior art must

provide motivation, in some manner, for the artisan to take the required steps to arrive at the claimed invention.

The claimed invention is not, however, primarily concerned only with a method using supercoiled DNA for analyzing DNA repair by excision and resynthesis, but instead provides a new type of assay which has been designed so as to be as specific as possible to the excision and resynthesis DNA repair capacities of the biological medium being tested and one of the most important aspects of the claimed method is that DNA repair mechanisms associated with strand breaks and nuclease activity are not measured. This specificity is achieved using supercoiled DNA, but, in accordance with the present invention, the artisan at the time the present invention was made could not have been aware that using this type of DNA would solve the technical problem overcome by the method according to claim 22. Also see claim 23, wherein the plasmids according to step (a) have an explicit double-stranded supercoiled form.

Clearly, neither cited reference even addresses the same problem addressed by the claimed method hence neither are in a position to provide a solution therefor.

The artisan would therefore have had no motivation to combine Calson et al. with that of '228 as the method of '228 does not suffer from the problem of a background of none excision and resynthesis repair events making the determination of the quantity of excision and resynthesis repair events difficult.

Moreover, the method according to present claim 22, is advantageously superior to the one described in '228 because although the exact mutations/lesions are not known for the mutated supercoiled plasmids, the target reagent (the plasmids in claim 22) contains a large number of mutation types in a wide variety of locations, and such a heterogeneous target reagent can more accurately quantify all the various excision and resynthesis repair capacities of the biological medium, than can a target reagent which consists of a large number of a single mutation type (as in '228). This advantage is neither disclosed nor suggested by either cited reference.

In fact, were the artisan to commence work starting with Calsou et al., seeking to perform a method for analyzing DNA repair by excision and resynthesis but excluding the effects of other types of DNA damage such as strand breaks and nuclease activity, with reference to '228 the artisan would surely have adopted the strategy described in '228, namely the use of predefined linear oligonucleotides which only contain mutations which are acted upon by excision and resynthesis systems. This is what one skilled in the art would be motivated to do from '228.

Thus, even assuming, *arguendo*, the artisan were to have combined the disclosures of '228 and Calsou et al., the artisan would have been motivated to use predefined linear oligonucleotides which contain only mutations which are acted upon by excision and resynthesis systems. The artisan would have had no motivation to use the method of present claim 22 using supercoiled DNA.

Further detailed comments concerning the combination of Calsou et al. and '228 are provided below.

As explained on page 1 paragraphs [0012] to [0020] of the present specification, the DNA excision and resynthesis repair systems of an organism, i.e., processes which are mediated by the removal of one or more improper base from a DNA molecule, contain two main pathways Base Excision Repair (BER) and Nucleotide Excision Repair (NER). BER and NER mainly act through different enzymes upon different types of mutation types, with BER acting to repair damage to a single base such as when the base becomes oxidised, alkylated or deaminated, and NER acting to repair larger damage such as base pair dimer formation and 6-4 photoproducts. The main enzymes involved in BER are DNA glycosylases and AP endonucleases; the main enzymes involved in NER are XPA, XPB, XPC, XPD, XPE, XPF, XPG, ERCC1, RPA, Rad23, CSA and CSB.

Calsou et al., as indicated in the title thereof, relates to Nucleotide Excision Repair (NER) and

induces mutations in the target DNA molecule using UV-C (page 27602 1<sup>st</sup> column 2<sup>nd</sup> paragraph 'Preparation of Plasmids'). UV-C predominantly causes mutations such as nucleotide dimers.

'228 relates to Base Excision Repair (BER) and therefore the target DNA molecules include mutations such as oxidised bases like 8-oxoguanine (see pages 5 paragraph [0076]).

These two cited references, '228 and Calsou et al., therefore, relate to methods of assaying different types of mutation repair mechanisms. Given the differences in mutation specificity shown by the NER and BER mechanisms, the skilled artisan would have been even further dissuaded from combining the teaching of these documents in the way the examiner has suggested.

The present inventors have shown for the first time that a single reaction upon assorted DNA targets can be used to assay NER and BER activity. For instance, in the same reaction a supercoiled DNA target treated with UV-C and a supercoiled DNA target treated with endoperoxide DHPNO<sub>2</sub>, can be positioned within one or more of the zones (A<sub>1</sub> to A<sub>x</sub>). These different supercoiled DNA targets will contain different mutations (amongst others, nucleotide base dimers in the case of the UV-C treated DNA target and oxidised bases in the case of the endoperoxide DHPNO<sub>2</sub> treated DNA target). Therefore, by monitoring the incorporation of label into these different targets, the NER and BER activities of the biological sample can be determined simultaneously. Prior art assays of BER activity utilize short oligos which include mutations targeted by BER such as those in '228. Attached to this response, with an Information Disclosure Statement (IDS) is a publication which illustrates this point, i.e. Kreklau et al., Nucleic Acids Res. 2001 Jun 15;29(12):2558-66, which describes another assay of BER and uses defined oligos containing ethanoadenine (an oxidised base) targeted by BER. Clearly, there is no cited prior art of record suggesting this simultaneous global detection of excision and resynthesis repair, and Kreklau et al. clearly indicates why.



That differently treated DNA targets can be combined in this way, in accordance with the claimed invention, so as to assess both the NER and BER capacity of a biological sample is neither disclosed nor suggested by any reference of record or consideration thereof. Instead, this methodology is only disclosed and claimed in the present application.

Thus, it is clear that '228 and Calsou et al., are non-analogous documents, using different DNA targets for different reasons. Hence, one skilled in the art would have had no reason to combine these references at the time the present invention was made. To suggest otherwise could only require impermissible *ex post facto*, or hindsight reconstruction, of the prior art in view of the claimed invention.

Hence, for all of the above reasons, this ground of rejection is believed to be unsustainable and should be withdrawn.

Claims 26-28 stand rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of Douki et al.

However, Douki et al merely disclose the use of HPLC coupled to mass spectrometry and, hence, this reference fails to correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claim 30 stands rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of MPEP 2144.05.

However, MPEP 2144.05 is merely cited as stating that “ optimizing concentrations is obvious”. See page 7 of the Official Action.

Thus, MPEP 2144.05 fails to correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claims 34-36 stand rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of Chiu et al.

However, Chiu et al. merely describe using an epoxy group to treat a glass slide, and fails to correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claims 32 and 37 stand rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of Zierdt et al.

However, Zierdt et al. merely disclose the use of a buffer containing phosphate and Tween 20.

However, this fails to correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claims 39 and 40 stand rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of Gelfand et al.

However, Gelfand et al. merely disclose the use of FRET to compare a reference DNA molecule to a DNA molecule which contains a lesion, which disclosure fails to correct the deficiencies of the two previously cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claims 45 and 46 stand rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of MPEP 2144.05.

However, even if optimizing time of incubation were considered to have been obvious to the artisan at the time the claimed invention was made, this would fail to correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claim 48 stands rejected under 35 USC 103(a) as being unpatentable over '228 and Calsou et al. in further view of Yershov et al.

However, Yershov et al. merely disclose DNA deposition by robot on an array. It is not seen how this disclosure would correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Claim 49 stands rejected under 35 USC 103(a) as being unpatentable under 35 USC 103(a) over '228 and Calsou et al. in further view of Randerath et al.

However, Randerath et al. is merely cited as disclosing labeling triphosphate with <sup>32</sup> P.

Clearly, this disclosure fails to correct the deficiencies of the previously two cited references.

Hence, this ground of rejection is unsustainable and should be withdrawn.

Finally, Applicant respectfully invites the examiner to contact the undersigned attorney to discuss any remaining questions that the examiner may have in order to facilitate allowance of this application.

Accordingly, in view of all the above, it is believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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